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The helminth fauna of the greater horseshoe bat (*Rhinolophus ferrumequinum*) (Chiroptera: Rhinolophidae) on the territory of Serbia

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Summary. An analysis of the helminth fauna of the greater horseshoe bat was conducted for the first time on the territory of Serbia. The host sample consisted of 17 individuals of the greater horseshoe bat (*Rhinolophus ferrumequinum*) collected from 8 localities in Serbia. Fifteen individuals (88.2%) were found to be infected with helminth parasites. Five parasite species were identified: three digeneans (*Plagiorchis* sp., *Prosthodendrium longiforme*, *Lecithodendrium linstowi*) and two nematodes (*Strongylacantha glycirrhiza*, *Litomosa ottavianii*). A total of 108 helminth individuals were collected from the hosts. The species *S. glycirrhiza* had the highest prevalence of infection (76.5%) and was represented by the highest number of individuals (65). Infection with a single parasite species was most commonly observed.

Keywords: digenean, greater horseshoe bat, nematode, Serbia.

INTRODUCTION

Rhinolophus ferrumequinum (Schreber 1774) is the largest species of horseshoe bat in Europe, and is widely distributed in the Palearctic region (Dietz et al. 2009). It is a sedentary species that travels 20-30 km (rarely over 100 km) during migrations between its summer and winter shelters (Hutterer et al. 2005). On the territory of Serbia, it inhabits karst areas with mosaically distributed trees, shrubs, open areas and speleological objects. It finds shelter in natural and artificial subterranean refuges such as caves, tunnels, abandoned mines, underground passages in fortresses, basements and attic spaces in buildings in Vojvodina (Paunović et al. 2011). In Europe, its diet consists primarily of larger insects of the Lepidoptera, Coleoptera, Diptera and Hymenoptera orders (Benda et al. 2012).

Within the former Yugoslavia, in what is today Croatia, Barus and Daniel (1972) found the nematodes *Molinostrongylus alatus* and *Capillaria italica* in *Myotis myotis*, and *Strongylacantha glycirrhiza* in *Rhinolophus ferrumequinum*. In addition, Vaucher (1975) identified the digenean *Prosthodendrium chilostomum* in the bat *Myotis capaccini* from the Zagorska cave near Novi Vinodol. Similarly, in Slovenia, Brglez and Bidovec (1987) recorded the nematode *Molinostrongylus alatus* in *M. myotis*. However, to date, studies of bat helminth fauna have not been conducted in Serbia.

To address this, the aim of the present study was to report the first data on the helminth fauna of the greater horseshoe bat in Serbia.

MATERIALS AND METHODS

Seventeen individuals of the greater horseshoe bat (11 males and 6 females) were sampled from 8 localities in Ser-

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bia: Deliblato sands (2 individuals), Zasavica (1), Valjevo (2), Đerdap (2), Bor (3), Beljanica (2), Kučevo (3) and Boljevac (2). The animals were sampled from 2001 to 2009, as part of scheduled activities of the Museum of Natural History, and in accordance with Serbian legislation pertaining to nature conservation and protection. Capture and collection was primarily carried out using mist nets and harp traps placed at entrances or inside various shelters such as caves. Additionally, mist nets of appropriate size (16 mm) were placed in different types of habitats.

Helmintological analysis was conducted on the bats, using standard methods. Digeneans were first soaked in distilled water and then dyed with borax carmine. The parasites were then dehydrated in a series of ethanol solutions of increasing concentrations. They were kept for 15 minutes in 70%, 80% and 90% ethanol, and an additional 5 minutes in 95% ethanol. The parasites were then transferred in cedar wood oil for illumination, and after 24 hours were mounted in Canada balsam on microscope slides. Nematodes were illuminated in lactic acid and observed as native slides.

Identification of parasites was carried out according to identification keys (Sonin 1975; Bray et al. 2008; Anderson et al. 2009) and data from selected articles (Matskási 1967; Esteban et al. 1999). The hosts are kept at the Museum of Natural History in Belgrade, Serbia. Parasites are stored in the zoological collection of the Department of Biology and Ecology of the Faculty of Sciences in Novi Sad, Serbia.

RESULTS

Of the 17 examined individuals of the greater horseshoe bat, 15 (88.2%) were found to carry helminth infections. One hundred and eight parasite individuals belonging to five species were found in the analysed hosts (Table 1). Four species were found in the intestine: *Plagiorchis* sp., *Prosthodendrium longiforme* (Bhalerao 1926), *Lecithodendrium linstowi* (Dollfus 1931) and *Strongylacantha glycirrhiza* (van Beneden 1873). The roundworm *Litomosa ottavianii* (Lagrange and Bettini 1948) was found on the gastric serosa.

The most commonly found species was *S. glycirrhiza* (Fig. 1), which was recovered from 13 host individuals (76.5%). The least frequent species was *P. longiforme* (5.9%). *Strongylacantha glycirrhiza* was also dominant with regards to number of individuals (65). Ten hosts were infected with only one helminth species and three hosts carried two parasite species, with 3 being the highest number of helminth species per host individual. Three different combinations were noted (*L. linstowi*-*S. glycirrhiza; Plagiorchis* sp.-*S. glycirrhiza* and *P. longiforme*-*S. glycirrhiza*), each one present in a single host. Two bats carried three helminth species: *Plagiorchis* sp.-*S. glycirrhiza*-*L. ottavianii* and *L. linstowi*-*S. glycirrhiza*-*L. ottavianii*. The maximum number of helminth individuals per host ranged from 1 to 29.

DISCUSSION

The present study represents the first analysis of bat helminth fauna conducted in Serbia. Thus, all of the species noted here represent new findings within the country. However, similar studies of this type have been carried out in selected European countries such as Hungary (Matskási 1967; Mészáros 1971), Spain (Esteban et al. 1991, 1999), Italy (Ricci 1995) and France (Bain 1966).

Five helminth species were identified: *Plagiorchis* sp., *Prosthodendrium longiforme*, *L. linstowi*, *S. glycirrhiza* and *L. ottavianii*. According to available data, digeneans and roundworms are frequently encountered in populations of horseshoe bats (Esteban et al. 1991, 1999; Ricci 1995). Esteban et al. (1991) noted that the high degree of helminth presence in chiropterans is caused by factors such as lifestyle, habitat use and diet. According to the same authors, the species *S. glycirrhiza* is a geohelminth, a nematode species with a direct life cycle. On the other hand, *L. ottavianii* is a diheteroxenous nematode (biohelminth) (Esteban et al. 1991). Mature females of this species produce microfilariae in the definitive host, which are then ingested by haematophagous insects which feed on the bats' blood (Bain 1966; Mészáros 1971).

Individuals of the genus *Plagiorchis* were measured, and results were comparable with data reported in previous studies (Lord et al. 2010). Body length, sucker positions and size of reproductive organs are in accordance with the description of *Plagirochis koreanus* given in the aforementioned paper. However, since genetic data was not analyzed in our study, we report our finding only as *Plagiorchis* sp. Further studies

Table 1. Helminth species found in the greater horseshoe bat (*Rhinolophus ferrumequinum*) on the territory of Serbia.

Org	Species	N par	N host	P%	Min-Max
Int	*Plagiorchis* sp.	5	2	11.8	1-4
	Prosthodendrium longiforme	8	1	5.9	8
	Lecithodendrium linstowi	23	3	17.7	4-11
	Strongylacantha glycirrhiza	65	13	76.5	1-17
GS	*Litomosa ottavianii*	7	3	17.7	1-5
	Total	108		88.2	

Org – organ; Int – intestine; GS – gastric serosa; N par – number of parasite individuals; N host – number of infected hosts; P% - prevalence of infection; Min-Max – minimum-maximum range of infection intensity.

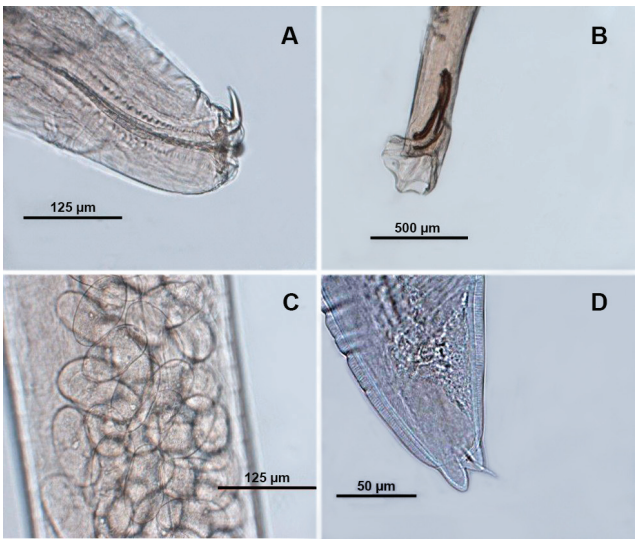

Fig. 1. *Strongylacantha glycirrhiza*. **A**, male, anterior region; **B**, male, caudal region; **C**, female, eggs; **D**, female, caudal region

on the subject in Serbia should incorporate genetic methods of species identification.

Plagiorchis species are widely found in numerous bat species in Europe. Ricci (1995) reported the presence of *Plagiorchis* in eight host species in Italy, including *Rhinolophus ferrumequinum*, *R. hipposideros*, *R. euryale*, as well as *Miniopterus schreibersii*, *Myotis mystacinus*, *M. daubentonii*, *M. capaccinii* and *Pipistrellus kuhlii*. Matskási (1967) found the species *Prosthodendrium longiforme* in the greater horseshoe bat and nine other bat species. *Lecithodendrium linstowi* is the most frequently encountered digenean species in European bats. Esteban et al. (1991, 1999) identified it from *R. ferrumequinum*, *R. euryale*, *M. schreibersii* and *Tadarida teniotis*. In Italy, this species was found in *R. ferrumequinum*, *R. euryale*, *R. hipposideros*, *Miniopterus schreibersii*, *Myotis capaccinii* and *P. kuhlii* (Ricci 1995). The roundworm *L. ottavianii* was reported from *M. schreibersii* and *R. ferrumequinum* in France (Bain 1966). In Spain, other authors (Esteban et al. 1991, 1999) found *L. ottavianii*, as well as *Rhinolophus euryale* and *R. mehelyi*, in the same two species. Thus, all of the parasite species noted above can be said to have a wide distribution, both in a geographical sense and with regard to the number of hosts they infect (Chiriac and Barbu 1973; Demidova and Vekhnik 2004; Kirillova et al. 2007; Shimalov et al. 2011; Lord et al. 2012; Kluwak et al. 2013).

On the other hand, *Strongylacantha glycirrhiza* is an oligoxenous species characterized by a narrow host specificity. Specifically, it is a parasite of *Rhinolophus* bats and is even used as an indicator in taxonomy (Esteban et al. 1999). Findings reported in other studies support this claim: *S. glycirrhiza* was identified from *R. ferrumequinum* and *R. hipossideros* in Hungary (Mészáros 1971), and only in *R. ferrumequinum* in Spain (Esteban et al. 1991, 1999).

All of the helminth species reported in the present study are common and expected parasites of the greater horseshoe bat, with the territory of Serbia representing a newly confirmed area of their distribution. The data presented here thus can be considered an early contribution to the study of chiropteran helminth fauna in Serbia, opening new fields of research from faunistic, epizootiologial and biocoenological aspects, and providing information on the biodiversity of local and regional animal communities.

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